BARD Queensland research Grant
Final Scientific Report

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Project Title: Resistance to *Tomato yellow leaf curl virus* (*TYLCV*) in tomato: molecular mapping and introgression of resistance to Australian genotypes

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Keywords: DNA markers, resistance genes, chromosome 4, *SlNAC1*.

Abbreviations: ToLCV= *Tomato leaf curl virus*, QTLs= quantitative trait loci, cM= centi Morgan, ssDNA= single strand DNA.

Budget: IS: $134,500 US: $125,000 Total: $259,500

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Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Publication Summary (numbers)

	Joint IS/Queensland authorship	Queensland Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged			1	1
Submitted, in review, in preparation				
Invited review papers				
Book chapters				
Books				
Master theses			1	1
Ph.D. theses				
Abstracts			1	1
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From Queensland to Israel	From Israel to Queensland	Together, elsewhere	Total
Short Visits & Meetings		1		1
Longer Visits (Sabbaticals)				

Description Cooperation: The collaboration between the Queensland and Israel was mainly underlined by the exchange of genetic material and DNA markers between the two countries. This genetic material and the DNA markers developed in the course of this study are being used in classical breeding in Israel and in Queensland and in map based cloning of genes encoding resistance in Israel.

Patent Summary (numbers)

	Israeli inventor only	Queensland inventor only	Joint IS/Queensland inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

Tomato yellow leaf curl virus (TYLCV) is one of the most devastating viruses of cultivated tomatoes. Although first identified in the Mediterranean region, it is now distributed world-wide. Sequence analysis of the virus by the Australian group has shown that the virus is now present in Australia. Despite the importance of the disease and extensive research on the virus, very little is known about the resistance genes (loci) that determine host resistance and susceptibility to the virus. A symptom-less resistant line, TY-172, was developed at the Volcani Center which has shown the highest resistance level among all tested varieties. Preliminary results show that TY-172 is a good candidate to confer resistance to both TYLCV and to *Tomato leaf curl virus* (ToLCV) in Queensland conditions. Furthermore, Segregation analysis has previously indicated that the resistance is determined by 2-3 genes. In this proposal we aimed to substantiate that TY-172 can contribute to resistance breeding against TYLCV in Queensland, to develop DNA markers to advance such resistance breeding in both Israel and Queensland, and to exploit these markers for resistant breeding in Australian and Israeli lines. To map quantitative trait loci (QTLs) controlling TYLCV resistance in TY172, appropriate segregating populations were analyzed using 69 polymorphic DNA markers spanning the entire tomato genome. Results show that TYLCV resistance in TY172 is controlled by a previously unknown major QTL, originating from the resistant line, and four additional minor QTLs. The major QTL, termed *Ty-5*, maps to chromosome 4 and accounts for 39.7-to-46.6% of the variation in symptom severity among segregating plants (LOD score: 33-to-35). The minor QTLs, originated either from the resistant or susceptible parents, were mapped to chromosomes 1, 7, 9 and 11, and contributed 12% to the variation in symptom severity in addition to *Ty-5*. Further analysis of parental lines as well as large F_1, BC_1F_1, F_2 and BC_1F_2 populations originating from crosses carried out, in reciprocal manner, between TY172 and the susceptible processing line M-82 (LA3475) during spring-summer 2010, indicated that: **(1)** the minor QTLs we have previously identified are in effect not reproducible, **(2)** *Ty-5* alone can yield highly resistant plants with practically no extra-chromosomal effects, and **(3)** the narrow-sense heritability estimate of resistance levels, attributed to additive factors responsive to selection, does not significantly deviate from 1. All of these results point to *Ty-5* as the sole resistance locus in TY172 thus significantly increasing the likelihood of its successful molecular dissection.

The DNA markers developed during the course of this study were transferred together with the TY172 genotype to Queensland. TY172 was crossed to a panel of Australian genotypes and the resulting populations were subjected to segregation analysis. Results showed that resistant locus, *Ty-5*, is highly reproducible in the Australian conditions as well. The Australian group was also able to make improvements to the marker assays by re-designing primer pairs to provide more robust PCR fragments. The *Ty-5* locus has now been introgressed into elite Australian germplasm and selection for TYLCV resistance has begun.

Cumulatively, our results show that *Ty-5* can be effectively used, together with the TY172 genotype to expedite TYLCV resistance breeding and improve our understanding of the genetics that underline the response of tomato to TYLCV. Contributions to agriculture include: **(1)** the development of tools for more efficient resistance breeding, allowing the incorporation of resistance to local tomato varieties in Australia, Israel and elsewhere; and **(2)** establish a solid framework for a future attempt to clone the genes that encode such resistance. The latter will enable to decipher the resistance mechanisms that could be applied to other geminiviruses in tomato and possibly in other plant species.

Achievements

Molecular analysis of TY172, carried out by the Israeli group (Anbinder et al., 2009 in the Appendix), showed that TYLCV resistance in TY172 is controlled by a major QTL, and four additional minor QTLs. The major QTL, termed *Ty-5*, was mapped to chromosome 4 and accounts for 39.7-46.6% of the variation in symptom severity among segregating plants (LOD score 33-35, depending upon population) and appeared to be sufficient to alone confer high levels of resistance. Although minor QTLs were also discovered on chromosomes 1, 7, 9 and 11, they cumulatively contributed only 12% to the variation in symptom severity in addition to *Ty-5*. Further analysis of parental lines as well as large F_1, BC_1F_1, F_2 and BC_1F_2 populations originating from crosses carried out, in reciprocal manner, between TY172 and the susceptible processing line M-82 (LA3475) during spring-summer 2010, indicated that: **(1)** the minor QTLs we have previously identified are in effect not reproducible, **(2)** *Ty-5* alone can yield highly resistant plants with practically no extra-chromosomal effects, and **(3)** the narrow-sense heritability estimate of resistance levels, attributed to additive factors responsive to selection, does not significantly deviate from 1. All of these results point to *Ty-5* as the sole resistance locus in TY172 thus significantly increasing the likelihood of its successful molecular dissection.

More fine-tune mapping of *Ty-5* was carried out during the spring-summer 2010, exploiting markers derived sequenced BAC clones surrounding this locus on chromosome 4 (Fig. 1, in the Appendix). This mapping effort revealed that *Ty-5* maps to ~200 Kb introgression, residing at the telomeric region of chromosome 4. This introgression displays ~1 recombination event per 7 Kb in F_2 populations, and delimited at its upper border by *SlNAC1* gene which thus far displayed the highest R^2 and LOD-score (Anbinder et al., 2009 and Fig. 2 in the Appendix). Interestingly, *SlNAC1*, encoding a member of the NAC-domain protein family, was previously implicated in the replication of the tomato-infecting begomovirus, ToLCV, by interacting with the viral replication enhancer protein (REn) (Selth et al., 2005; Plant Cell 17:311-325). Selth et al. (2005) further showed that ToLCV and TYLCSV induce *SlNAC1* expression specifically in infected susceptible cells, and that this up-regulation requires REn. Also, in a transient ToLCV replication system, over-expression of *SlNAC1* resulted in a substantial increase in viral DNA accumulation. Together, these results suggest that *SlNAC1* plays a pivotal role in the process by which REn enhances replication of ToLCV and possibly other begomoviruses, including TYLCV. Indeed, one of the main events that was thus far clearly associated with TYLCV infection in resistant TY172 plants was reduced

TYLCV ssDNA accumulation at the site of inoculation in comparison to susceptible lines which can be attributed to *SlNAC1* (Segev et al., 2004; *4th International Geminivirus Symposium*, ABSTRACT W1, Cape Town, South Africa.). Moreover, recent analysis of fully isogenic resistant (16R) and susceptible (16S) lines originating from TY199, another TYLCV resistant line developed from *S. peruvianum* at Volcani Center, demonstrated that *SlNAC1* is transcriptionally up-regulated specifically in the susceptible line under TYLCV inoculation, but not in the resistant line (Fig. 3 in the Appendix). In addition, sequence analysis of *SlNAC1* in TY172 and two susceptible lines, revealed a relatively conserved Tyrosine^{212} to Cysteine substitution in TY172 and also: three single nucleotide polymorphisms (SNPs) in the promoter region and two SNPs in the first intron of the gene (Levin I and Lapidot M, unpublished). All of these results molecularly, structurally and functionally implicate *SlNAC1* in the slower TYLCV infection progression in TY172, suggest that at least part of the resistance to TYLCV can be directly attributed to inhibition of the TYLCV-induced up-regulation of *SlNAC1* transcription, and merit its further study.

The DNA markers developed during the course of this study were transferred together with the TY172 genotype to Queensland. TY172 was crossed to a panel of Australian genotypes and the resulting populations were subjected to segregation analysis. Results showed that resistant locus, *Ty-5*, is highly reproducible in the Australian conditions as well.

Complete genome (2781 nt) of four isolates of TYLCV has been sequenced in Ausralia. These originated from the Brisbane area (two isolates) and one each from Bundaberg and the Lockyer Valley. All sequences were ≥ 98.8% identical and were closely related to isolates from China and Japan (> 98% identical).

RFLP analysis on a total of 13 TYLCV isolates, using Dde I And Bam HI, revealed two variants. One was a subset of some Bundaberg isolates only. The other was due to the presence of a defective mutant (approx. half genome size – 1.3 kb) found in a single Brisbane isolate.

Isolates of Tomato Leaf Curl Australia virus from Darwin and north Queensland were also amplified by PCR and subjected to RFLP analysis using Dde I. Both isolates produced patterns distinct from TYLCV.

The Australian group has also established a whitefly rearing and inoculation facility. Both healthy and infectious (TYLCV) colonies of silverleaf whitefly (*Bemisia tabaci* Biotype B) have been established at Indooroopilly. The Infectious colony has been used successfully to test inoculate Israeli tomato differential resistance lines. Israeli resistant lines 1494 F_1, PRT 352, PRT 354, 5152, 5153 and TY197, and the commercial lines Pintero, Felicity and

Tygress, which contain the TY-1 resistance gene, were resistant to the Australian isolate of TYLCV. The purportedly resistant cultivar Cassablanca was susceptible. The three Australian commercial cultivars tested were susceptible and showed strong disease symptoms.

The inoculation facility has also been used to establish that French bean (*Phaseolus vulgaris* cv. Bountiful), capsicum (*Capsicum annuum* cv. Yolo wonder*)* and *Datura strammonium* were susceptible. Datura and bean showed strong leaf curling, but capsicum was symptomlessly infected. Cotton (*Gossypium hirsutum*) and eggplant (*Solanum melongena*) were not infected.

Cumulatively, our results show that *Ty-5* can be effectively used, together with the TY172 genotype to expedite TYLCV resistance breeding in Australia and improve our understanding of the genetics that underline the response of tomato to TYLCV. Contributions to agriculture include: **(1)** the development of tools for more efficient resistance breeding, allowing the incorporation of resistance to local tomato varieties in Australia, Israel and elsewhere; and **(2)** establish a solid framework for future attempt to clone the genes that encode such resistance. The latter will enable to decipher the resistance mechanisms that could be applied to other geminiviruses in tomato and possibly in other plant species.

Details of cooperation

The collaboration between the Queensland and Israeli groups led to the confirmation that *Ty-5* can be effectively used to breed TYLCV resistant cultivars in both countries. This conclusion was achieved by the exchange of genetic material and DNA markers between the two countries. The genetic material and DNA markers exchanged are being used in classical breeding in Israel and in Queensland and for map based cloning of the gene (or genes) conferring resistance at the *Ty-5* locus in Israel.

List of publications

Anbinder I, Reuveni M, Azari R, Paran I, Nahon S, Shlomo H, Chen L, Lapidot M, Levin I (2009) Molecular dissection of *Tomato leaf curl virus* resistance in tomato line TY172 derived from *Solanum peruvianum*. Theor Appl Genet 119:519-530.

Anbinder I (2009) Genetic characterization of resistance to *Tomato yellow leaf curl virus* in tomato plants. M. Sc. Thesis Submitted to the Faculty of Agriculture, Food & Environmental Quality Sciences of the Hebrew University of Jerusalem for the degree of Master of Science.

Levin I, Karniel U, Anbinder I, Reuveni M, Nahon S, Shlomo H, Chen L, and Lapidot M. (2010) Molecular dissection of *Ty-5*, a *Tomato yellow leaf curl virus* resistance locus in the tomato line TY172 derived from *Solanum peruvianum*, the 6th International Symposium on Geminivirus, Guanajuato, Mexico.